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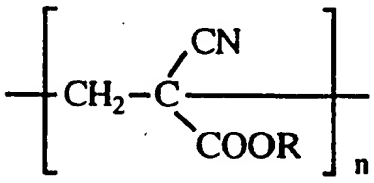

(I)

(57) Abstract

Nanocapsules typically having a diameter in the range of 20-150 nm are provided and consist of a polymeric shell formed of a surface active poly(alkyl cyanoacrylate) material arranged in one or more layers. The polymeric shell can be composed of a surface active poly(alkyl 2-cyanoacrylate) having general formula (I), wherein R is $-CH_2-(-CH_2-)_mCH_3$, $-CH_2-(-CH_2-)_mCOOH$, $-CH_2-(-CH_2-)_mCOOR'$, or $-CH_2CH_2-O-(-CH_2CH_2-O-)_mR'$; R' is $-CH_3$, $-CH_2-(-CH_2-)_mCH_3$, $-C-(-CH_3-)_2-CH_2-C(CH_3)_3$, or -O-Ar; m has a value of from 0 to 20; and n has a value of from 1 to 20, formed by the interfacial polymerisation of self-arranged micelles of the cyanoacrylate monomers. The nanocapsules can be used to encapsulate a wide range of water soluble and water insoluble active agents in high yield for delivery to a target system or locus, for example, drugs for use in therapy or prophylaxis.

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Description

Small diameter nanocapsules, process for their preparation and applications thereof

Technical Field

This invention relates to small diameter (20-150 nm) nanocapsules, a process for their preparation and the use of the nanocapsules so prepared for the delivery of active agents.

Background Art

Nanocapsules are examples of nanoparticles which are used *inter alia* as drug carrier systems. Nanoparticles are either small solid spheres (nanospheres) or small capsules (nanocapsules) formed of a central cavity surrounded by a shell or wall.

Nanoparticles can be used to achieve controlled delivery of drugs and also to deliver drugs to specific target cells.

Thus, nanoparticles are used *inter alia* to administer labile active agents or toxic anti-tumour agents to a subject. Conventionally, nanoparticles are administered by the intramuscular or intravenous route and are transported into the epithelial cells, blood cells and liver cells by phagocytosis. Alternatively, the nanoparticles are degraded by chemical and/or enzymatic processes in the blood.

Nanoparticles, such as poly(ethyl cyanoacrylate) particles, can be broken down by the Kupffer cells of the liver resulting in release of the active agent.

The distribution and fate of nanoparticles in the body after administration thereto depends on nanoparticle diameter. Small diameter nanoparticles (50-100 nm) are broken down by epithelial cells of the blood vessels. Middle size nanoparticles (100-400 nm) are

mainly broken down by blood cells. Nanoparticles with a diameter larger than 300-800 nm are mainly broken down by the Kupffer cells of the liver. Super small diameter nanoparticles (10-30 nm) are able to penetrate the blood-brain barrier and to deliver drugs into the brain.

Known types of nanoparticles (Review Article; Couvreur, P. and Vauthier, C. (1991), Journal of Controlled Release *17*, 187-198) include poly(butyl cyanoacrylate) nanocapsules (Al Khouri Fallouh, N. (1984); Pharm. Ph.D., *No. 207*, Paris XI) and poly(isobutyl cyanoacrylate) nanocapsules (Al Khouri Fallouh, N. (1986); International Journal of Pharmaceutics *28*, 125-132). The latter paper describes a process for the formation of nanocapsules by a mechanism which is described as being probably that of interfacial polymerisation resulting from the dispersion of an alcoholic solution of isobutyl cyanoacrylate and oil in water. This process involves the use of two immiscible phases and the nanocapsules so formed are oil-filled and can be used to entrap lipophilic substances. Only middle size nanocapsules with an average diameter of 200-300 nm can be obtained by the process described.

Damgé, C. *et al.* (Diabetes (1988) *37*, page 246) describe poly(alkyl cyanoacrylate) nanocapsules as a drug carrier for insulin. The rate of encapsulation of insulin was found to be 54.9%. The nanocapsules were prepared by the method of Al Khouri Fallouh N. (1984) *supra* and as such the insulin was encapsulated in a lipophilic phase.

There is a need for stable, aqueous- and non-aqueous-filled nanocapsules so as to extend the range of active substances that can be delivered by means of such nanocapsules. There is also a need for stable small diameter nanocapsules capable of delivering active agents into target cells *via* administration to the human or animal body, including the vascular system and the brain.

EP-A 0 274 961 describes the preparation of middle size nanocapsules (100-400 nm) from dispersible colloidal systems. It is

indicated that a wide range of substances (B) which are soluble or dispersible in a given solvent can be encapsulated by the process described. However, the process as described will result in a core of an organic solvent, an oily phase or a particulate substance. This will limit the nature of the active agent that can be encapsulated. For example, many of the organic solvents or solvent systems described would affect the stability of biologically active agents, such as peptides and proteins, and would be likely to lead to denaturation thereof and loss of pharmacological activity. The document does not describe the formation of aqueous-filled middle size nanocapsules nor small diameter nanocapsules.

Other examples of microcapsular drug carrier systems include liposomes which are small phospholipid based vesicles having an aqueous core. Liposomes obtained by crosslinking of lecithin have lipoidic walls structurally related to those of biological membranes and as such have a shell defined by a molecular bi-layer.

Using lecithin it is possible to synthesize small diameter vesicles (20-50 nm). However, liposomes are difficult to manufacture on an industrial scale. It is found that entrapped agents desorb very rapidly from liposomes into the bloodstream, such that drug delivery to phagocytic cells is not achieved.

This desorption of active agent and hence the limited stability of liposomes is due principally to the rapid hydrolysis thereof by blood enzymes.

Moreover, the entrapment levels of active agent achieved with liposomes are low (see Al Khouri Fallouh, N. (1986) *supra*).

Ways of improving drug delivery, so as to achieve better bioavailability and pharmacokinetics are constantly being sought, especially for active agents which are subjected to rapid degradation following administration.

Oral administration is one of the modes of administering drugs which has the greatest degree of patient compliance and thus ways are constantly being sought of formulating active agents for administration by the oral route which it has not hitherto been possible to administer by that route.

The low hydrolytic stability of liposomes means that they cannot be used for the delivery of active agents by the oral route. Enzymes of the gastrointestinal tract rapidly destroy liposomes following administration *via* the oral route with release of active agent, so that uptake of active agent from the intestinal tract into the bloodstream is not realised.

Disclosure of Invention

The invention provides nanocapsules comprising a polymeric shell formed of a surface active poly(alkyl cyanoacrylate) material arranged in one or more layers.

The nanocapsules according to the invention are stable and can be used to entrap effective amounts of an active agent. It is possible to achieve a degree of encapsulation of 75% or higher with the nanocapsules according to the invention.

By polymer herein as regards the polymeric shell is meant any suitable polymer according to the I.U.P.A.C. definition of polymer.

The polymeric shell of the nanocapsules according to the invention is made up of polymer chains typically of the order of 10 or more monomer units. The polymer shell formed has the ordered arrangement of a mono-, bi-, tri-, or polymolecular layer typical of a liposome.

Preferably, the nanocapsules in accordance with the invention have a diameter in the range 20-150 nm.

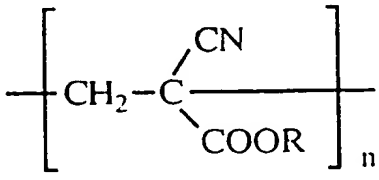
The size of the nanocapsules is determined by the type of monomer used and by the method of preparation as hereinafter described.

The presence of one or more ordered layer(s) in the polymeric shell follows from the size of the nanocapsules produced and may be confirmed by electron diffraction.

The nanocapsules according to the invention are primarily intended for use in the delivery of active agents to the human or animal body, including delivery for the purposes of medical diagnosis involving imaging. However, the nanocapsules according to the invention are not limited to such use and will also find application in agriculture, in cosmetics for delivery of a wide variety of active agents including fragrances, the food industry and other areas of technology to which their properties are adapted to provide a desired effect. For example, the nanocapsules according to the invention are ideally suited for the encapsulation and subsequent delivery of systemic fungicides, herbicides and pesticides and plant growth controlling agents to plants.

Thus, typically an active agent is contained in the aqueous- or non-aqueous phase contained in the core.

An especially preferred polymeric material for the nanocapsular shell is a poly(alkyl cyanoacrylate) material, more especially a surface active poly(alkyl 2-cyanoacrylate) having the general formula:



wherein

R is $-CH_2-(CH_2)_m-CH_3$, $-CH_2-(CH_2)_m-COOH$, $-CH_2-(CH_2)_m-COOR'$, or $-CH_2CH_2-O-(CH_2CH_2-O)_m-R'$;

R' is $-CH_3$, $-CH_2-(CH_2)_m-CH_3$, $-C(CH_3)_2-CH_2-C(CH_3)_3$, or $-O-Ar$;

m has a value of from 0 to 20; and
n has a value of from 1 to 20.

According to a preferred method, the nanocapsules are formed by interfacial polymerisation of self-arranged micelles of surface active cyanoacrylate monomers in an aqueous phase as hereinafter described.

The active agent encapsulated in the nanocapsules according to the invention is any water soluble or water insoluble active agent, including naturally occurring substances and synthetic analogues thereof.

Given that the active agent is dissolved or dispersed in an aqueous or non-aqueous phase in the core of the nanocapsule, the stability thereof is maximised.

Especially preferred active agents for encapsulation in the nanocapsules according to the invention are water soluble active agents.

Such preferred active agents include amino acids, peptides and polypeptides. Such active agents include hormones, hormone release factors, cytokines, encephalins, blood factors and products including enzymes and antibodies, and other active agents which are susceptible to degradation and/or modification by proteolytic and other enzymes before exerting their effect, especially if administered by the oral route. The latter type of active agents also includes anti-tumour agents, antibiotics, opiates such as apomorphine, dopamine, serotonin and

other agents active on the central nervous system, and steroid hormones such as progesterone and testosterone.

When the active agent is an antibody, the antibody can be a monoclonal or polyclonal antibody.

The nanocapsules according to the invention are also especially suitable for the encapsulation and subsequent delivery of immunomodulating agents, for example, cyclosporin.

The nanocapsules according to the invention can also be used to encapsulate various vaccines.

It will be appreciated that the nanocapsules according to the invention can increase the bioavailability and efficacy of a wide range of water soluble active agents by protecting said agents from premature degradation in the gastrointestinal tract and the blood and allowing for a sustained release thereof.

The invention also provides an active agent delivery system comprising nanocapsules as hereinbefore described.

The nanocapsules according to the invention are stable and release their contents on degradation following administration to the target system or locus.

The nanocapsules according to the invention when intended to deliver an active agent for use in therapy or prophylaxis may be administered orally, parenterally or topically to the human or animal body. Following oral administration the nanocapsules traverse the gut wall and are taken up into the blood stream and the product is released on degradation of the nanocapsule shell or wall.

The nanocapsules are useful in delivering active agents to the blood stream by the oral route that are not normally suitable for administration by this route in traditional conventional pharmaceutical

formulations. The encapsulated product is protected from the harsh conditions of the gut, such that a significantly greater proportion of active agent is delivered to the bloodstream than would be possible by simple oral administration of the non-encapsulated active agent. For example, insulin, a protein, is normally given by intramuscular injection. If given orally it is normally degraded by the normal digestive processes of the gut and only a very small and variable proportion finds its way into the bloodstream. Insulin encapsulated by the method according to the invention can be given orally with minimal loss of pharmacological effect. Thus, there are major benefits for the patient both in terms of reducing stress and increasing convenience.

Suitable formulations of the nanocapsules according to the invention for administration by the oral route include capsules, dragées, elixirs, granules, lozenges, pellets, powders, suspensions and tablets. In the case of tablets care should be taken that the tabletting technique does not lead to any disruption of the nanocapsules and alteration of their release properties. Such tablets can be formulated for rapid disintegration in the gastric and/or intestinal juices, if required or, alternatively, coated so as to further delay the release of the active agent.

The nanocapsules can also be formulated as solutions or suspensions for injection intramuscularly, intravenously and subcutaneously. It is also possible to formulate the nanocapsules according to the invention in liquid form for administration by perfusion.

Further types of formulations according to the invention include nasal formulations, ocular agents, including slow release implants containing the nanocapsules, pessaries, suppositories, lozenges coated on one surface with a bioadhesive for use in the buccal cavity or formulations for administering an active agent sublingually.

The nanocapsules will generally be formulated in unit dosage form for administration or application in an amount and for a time prescribed by an attending physician.

A preferred method for preparing the nanocapsules according to the invention comprises interfacial polymerisation of a surface active cyanoacrylate monomer in the form of a colloidal solution composed of self-arranged micelles in an aqueous medium under polymerisation initiating conditions. Colloidal particles of an active agent to be encapsulated may serve as the initiator of polymerisation. However, polymerisation can also be spontaneous. The aqueous medium is preferably composed of a two phase aqueous system.

A single or mono-phase aqueous system for use in accordance with the invention is typically a solution physiologically isotonic in strength and comprises water or an aqueous solution of one or more water-soluble polymers and/or one or more water soluble salts.

A two phase aqueous system for use in accordance with the invention preferably comprises an aqueous colloidal solution of two or more water soluble immiscible polymers. Such water soluble immiscible polymers are known (see for example, Alberdsson, P.A. "Partition of cell particles and macromolecules", Wiley, International Scientific N.Y. (1971) pp 30-37).

The polymers are selected primarily on the basis of their compatibility and density. As regards the former criterion, there should be little or no affinity between the polymers, such that they do not form aggregates or interact unfavourably in solution.

The following are examples of suitable combinations of polymers:

Dextran sulphate and methylcellulose
Dextran sulphate and polyethyleneglycol

Dextran sulphate and polyvinylalcohol
Diethylaminoethyldextran and polyethyleneglycol
Dextran and polyvinylalcohol
Dextran and methylcellulose
Dextran and polyethyleneglycol
Dextran and Ficoll (Ficoll is a Trade Mark)
Dextran and oxypropyldextran
Dextran and a mixed polymer of ethylene oxide and propylene glycol such as Pluronic (Pluronic is a Trade Mark)
Dextran and polypropyleneglycol
Oxypropyldextran and polyethyleneglycol
Sodium carboxymethyldextran and polyvinylpyrrolidone
Dextran and chitosan
Dextran and dextran sodium sulphate

The formation of a stable two phase system also depends on the concentrations of the respective polymers in the solution. If the concentration of the polymers is below a critical level, then the two aqueous polymers will not separate into layers. The behaviour and characteristics of different polymers in combination must be determined empirically (see Alberdsson, P.A. (1971) *supra*).

The polymerisation initiating conditions preferably involve the use of an initiator of anionic polymerisation, which initiator is located within colloidal particles of a discrete phase or within a continuous phase. Examples of anionic polymerisation initiators include substances containing nucleophilic groups such as, for example, amines and thiols.

Generally, the continuous phase will be present in a large excess relative to the discrete phase, for example in a ratio of 100:1-50:1.

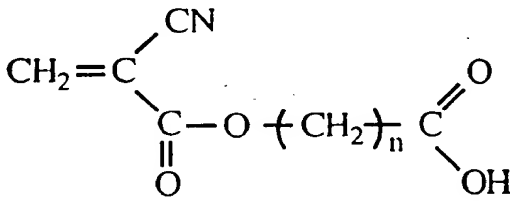
The interfacial micelles of cyanoacrylate monomer preferably self-arrange on the surface of colloidal particles of a discrete phase or within solution. To provide a cyanoacrylate monomer having the ability to self-arrange in an aqueous medium to form micelles surface

active cyanoacrylic monomers of the type hereinabove defined are used.

In order to provide cyanoacrylate monomers having surface active properties, hydrophilic or hydrophobic radicals are introduced into the cyanoacrylate molecule as hereinafter described.

The synthesis of surface active cyanoacrylate monomers for use in accordance with the invention is illustrated by the following preparation Examples.

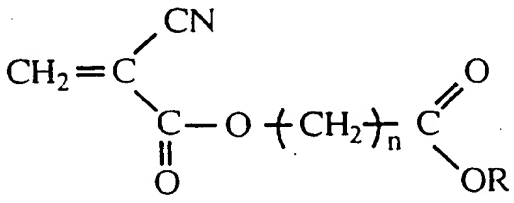
Suitable anionic type surface active alkyl 2-cyanoacrylates having an ionogenic moiety in the ester radical have the general formula:



wherein
n has a value of from 1 to 20.

Suitable nonionic surface active alkyl 2-cyanoacrylates having a hydrophilic moiety in the ester radical have one of the following formulae:

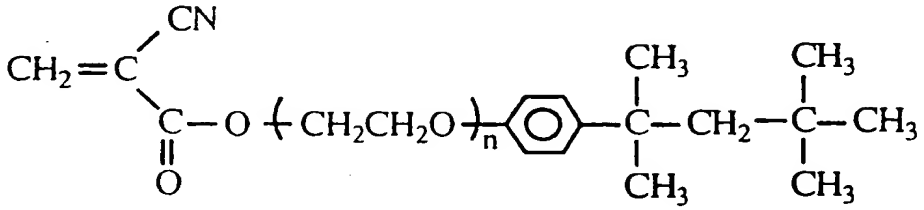
i)



wherein

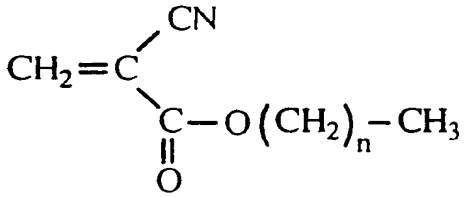
R is $-CH_3$, $-CH_2(CH_2)_mCH_3$, $-C(CH_3)_2-CH_2(CH_3)_3$, or $-O-Ar$; and

m and n each has a value of from 1 to 20;

ii) 

wherein

n has a value of from 1 to 20; and

iii) 

wherein

n has a value of from 0 to 20.

The principal stages in the encapsulation process are as follows:

Stage 1: The preparation of a continuous phase.

Stage 2: Polymerisation of a cyanoacrylate monomer to form capsules.

Encapsulation using a mono-phase aqueous system

In Stage 1, an aqueous solution is formed for use as a polymerisation medium for obtaining capsules. Preferably, an isoosmotic solution containing water soluble polymer and corresponding salts is used. An inhibitor of polymerisation, preferably cyanoacrylic acid, and a water soluble active substance are added to form a solution which is physiologically isotonic in strength.

In Stage 2, a surface active ester of cyanoacrylic acid is added with vigorous stirring, preferably using sonication. Micelles of monomer are formed in a continuous phase and then the monomer polymerises to form a solid shell of polymer. To increase the rate of polymerisation an initiator having a basic nature such as hydroxyl ion or heating to a temperature of up to 60°C is used. The active substance is encapsulated following polymerisation of the monomer to form solid micelles due to the difference in hydrophilicity inside and outside the micelles. Preferably the solid shell is composed of a mono- or bi-molecular layer of polymer.

Encapsulation using a two phase system

Encapsulation using a colloidal solution of a water insoluble active substance

The preparation of the continuous phase in the Stage 1, is similar to that for a mono phase aqueous system hereinabove described. However, the active agent is added in the form of a solution in an organic solvent, preferably an alcohol, or in the form of a suitable aqueous solution containing one or more solubiliser(s) or other additives.

In Stage 2, the size of colloid particles of the active agent is determined before addition of the monomer, and the colloid solution is sonicated if necessary. The surface active cyanoacrylate monomer is added with stirring and the micelles of monomer are formed on the surface of colloid particles of active substance. The solid shell is formed on the surface of colloid particles following polymerisation of the monomer to form a polymolecular layer of polymer. The surface of the colloid particles serves as the initiator of polymerisation while a bulk phase serves as a polymerisation inhibitor.

Encapsulation using a two phase aqueous solution

In Stage 1, a two phase aqueous system is formed containing an aqueous solution of two or more immiscible water soluble polymers capable of forming such a two phase system as follows. The polymers selected are dissolved in water, whereupon they settle into two layers, following equilibration of the respective polymers. This separation occurs primarily because the polymers are of different densities. If required, a further polymer which partitions selectively into one or other of the layers may be added during this stage, said further polymer having the capability to selectively concentrate a target active agent to be added in stage 2 and which it is desired to encapsulate in either one or the other phase of the two phase system.

The rate or time required for the separation of the layers depends on the choice of the individual polymers. Left to gravity alone, the separation can take from several hours to several days. Separation can be accelerated by centrifugation.

After separation, the two aqueous solutions, upper and lower, are decanted into separate flasks. At this point, two stable systems have been created in which the upper phase is equilibrated with lower phase and *vice versa*. Both phases are used in the creation of an emulsion in Stage 2.

Stage 2:

In this stage, an emulsion is formed between the two separated phases described above. Typically, the emulsion formed contains the two phases in a ratio of the order of 100:1. On emulsification, small droplets of the minor component form a discrete phase dispersed within a bulk or continuous phase of the major component which is present in excess. As both components in the emulsion have been mutually equilibrated in Stage 1, the droplets are relatively stable in the mixture and thus an emulsion can be formed.

The emulsion is formed by vigorous agitation such as that achieved by means of sonication or vortex mixing. The size of the droplets forming the discrete phase is primarily controlled by the degree and rate of agitation.

The active agent to be encapsulated is included in the emulsification process. The choice of upper or lower phase (created in Stage 1) to form the minor component in the emulsion is determined by the physical and chemical properties of the active agent. Indeed, such properties also influence the choice of polymers used in Stage 1. The droplets within the emulsion become encapsulated by the alkyl 2-cyanoacrylate added in the next stage - Stage 3. Accordingly, it is desirable to selectively concentrate the active agent inside the droplets. Thus the choice of upper or lower phase to form the droplets in the emulsion is primarily determined by the affinity of the active agent for the respective phases.

The method also allows for an initiator of polymerisation to be concentrated inside the droplets. In some instances, this may be the active agent itself. If this is not an initiator, however, this must also be added at this stage.

Stage 3:

In this stage, an alkyl 2-cyanoacrylate is added and the droplets are encapsulated following polymerisation at the droplet surface. Alkyl 2-cyanoacrylates polymerise on contact with an initiator of polymerisation. Nucleophilic chemical groups are good initiators of polymerisation. Conversely, acids, particularly strong acids, inhibit the polymerisation process. In the emulsion created in Stage 2, polymerisation in the bulk phase or continuous phase is inhibited by a low pH. This effect is reversed when the monomers encounter the initiator at the surface of the droplet, resulting in encapsulation by polymerisation of the droplet which includes the active agent.

Apart from the degree of agitation a number of other factors determine the size of the nanocapsules according to the invention. In general, the more rapid the rate of polymerisation, the lesser the degree of control over polymerisation and the greater the size of the nanocapsules formed.

Furthermore, the rate of polymerisation is inversely proportional to the size of the alkyl group in the alkyl cyanoacrylate monomer, so that the larger the alkyl group, the slower the polymerisation and hence the smaller the nanocapsules formed.

Also the lower the pH of the aqueous solution, the slower the rate of polymerisation and the smaller the size of the nanocapsules formed.

The rate of polymerisation is primarily controlled by pH and the size of the ester (alkyl) groups.

Best Modes for Carrying Out the Invention

The invention will be further illustrated by the following Examples.

In the following Examples the method according to the invention is exemplified by the encapsulation of a dye (DiI) and the peptide type hormone oxytocin.

Example 1

2-Cyanoacrylic acid ester of polyethylene glycol 4-tert-octylphenyl ether

A 500 ml flask was fitted with mechanical stirrer, thermometer, argon and sulphur dioxide inlet adaptors, dosing funnel protected with a drying tube, and Liebig condenser arranged for distillation. The flask was charged with 250 ml of anhydrous toluene, and 1 g of 2-cyanoacrylic acid was added to the boiling solvent with stirring and

sparging with argon. 20 ml of toluene/water azeotrope was removed by distillation and 2.2 g of phosphorus pentachloride dissolved in 50 ml of dry benzene was then added dropwise with stirring and constant removal of benzene by distillation. The mixture was stirred under reflux for 1 hour and then sparged with sulphur dioxide, while 100 ml of toluene containing by-product phosphorus oxychloride was distilled off to leave a residual colourless solution of 2-cyanoacryloyl chloride in toluene. A solution of 7.4 g of Triton-X100 (Triton is a Trade Mark) and 0.5 g of hydroquinone in 50 ml of benzene was then added dropwise to the 2-cyanoacryloyl chloride solution with stirring and constant removal of benzene by distillation. The mixture was refluxed during 1 hour, cooled, and solvent was distilled off in vacuum to give 8.1 g of a colourless oil which was washed with hot hexane to give 7.8 g of the 2-cyanoacrylate ester of polyethylene glycol 4-tert-octylphenyl ether. Elemental Analysis Calculated for $C_{38}H_{63}NO_2$: C 67.35%, H 9.30%, N 2.07%, Found C 66.7%, H 9.6%, N 1.9%, ^1H NMR in 1 : 1 C_6D_6 : $(CD_3)_2CO$ 0.75 (9H, s, $(CH_3)_3C$-), 1.37 (6H, s, $(CH_3)_2C$-), 1.77 (2H, s, CH_2), 3.62 (m, CH_2O-), 3.98 (t, J = 4 Hz, CH_2O-), 4.13 (t, J = 5 Hz, CH_2O-), 4.43 (m, 2H, CH_2OCO-), 6.08 (s, 1H, H-C=C-), 6.67 (s, 1H, H-C=C-), 6.88 and 7.33 (2d, each 2H, A_2B_2, J = 7.2 Hz, aryl) ppm.

Example 2

2'-Carboxyethyl 2-cyanoacrylate

9.8 g of 2-cyanoacrylic acid, 0.2 g of 4-toluenesulphonic acid and 0.1 g of hydroquinone were dissolved in 250 ml of anhydrous benzene in a 500 ml flask which had previously been washed with 10 % sulphuric acid and dried using acetone, and which was fitted with a stirrer, a thermometer, sulphur dioxide and argon inlet adaptors, a dosing funnel and a Liebig condenser arranged for distillation. The solution was sparged with sulphur dioxide and brought to reflux when a suspension of 9.9 g of 3-hydroxypropionic acid in 200 ml of benzene was added dropwise with continuous removal of benzene-water azeotrope by distillation. The mixture was heated with stirring and

sparging with sulphur dioxide until the benzene-water azeotrope ceased to appear, and was then refluxed for a further 30 minutes. The volume was reduced to 100 ml by removal of solvent by distillation. The residual colourless solution was cooled, filtered, and diluted with 500 ml of heptane to give 8.5 g of a solid which was collected. The solid was recrystallised from 1 : 1 benzene : heptane which had been saturated with sulphur dioxide to yield 6.51 g of 2'-carboxyethyl 2-cyanoacrylate.

Example 3

Preparation of hexadecyl 2-cyanoacrylate

Into a 0.5 litre flask fitted with mechanical stirrer, thermometer, argon inlet adaptor with a device for admitting a stream of gas under the surface of the reaction mixture, a dosing funnel protected with a Drierite drying tube, a Liebig condenser provided with a vacuum distillation adaptor and a receiver flask connected to a vacuum flask was charged 0.98 g (0.01 mole) 2-cyanoacrylic acid, 50 mg methylhydroquinone, 200 ml dry benzene and 100 ml dry toluene. A solution of 2.08 g (0.01 mole) phosphorus pentachloride in 50 ml of dry toluene was charged into the dosing funnel. While sparging with dry argon and stirring under reflux the phosphorus pentachloride solution was added dropwise. Following completion of the addition the reaction mixture was boiled for 15 minutes following which the reflux condenser was substituted by a Liebig condenser with a receiver and a calcium chloride drying tube and 200 ml of solvent were distilled off. At this point 2.42 g (0.01 mole) n-hexadecyl alcohol in 50 ml dry benzene was added from the dosing funnel while refluxing and stirring and sparging with dry argon. Following addition of the alcohol the mixture was boiled for one hour and then the solvent was distilled off to give 50 ml remaining which was cooled to 5°C and left overnight (17 hours), following which crystals of 2-cyanoacrylic acid had fallen out which were filtered off. The volatiles were removed by distillation under vacuum and the remaining solid recrystallised from hexane to give 1.57 g n-hexadecyl 2-cyanoacrylate (49% yield) solid; m.p. 51-

3°C [Elemental Analysis Calculated for $C_{20}H_{35}NO_2$ C = 74.8, H = 10.9, N = 4.4, Found C = 73.5, H = 11.1, N = 4.1].

^{1}H NMR δ6.24 (1H, s, CH_a=C-), 5.38 (1H, s, CH_b=C-), 3.89 (2H, t, J=5.8Hz, -CH_2OCO-), 1.32 (28H, m, $(CH_2)_{14}$-), 0.91 (3H, t, -CH_3) ppm. ^{13}C NMR C δ13.62 CH_3, 22.29 CH_3CH_2, 31.56 $CH_3CH_2CH_2$, 29.0$(CH_2)_{10}$, 25.27 CH_3 $(CH_2)_{12}$ CH_2, 27.95 ($CH_3(CH_2)_{13}CH_2$), 65.98 CH_3 $(CH_2)_{14}$ CH_2O, 113.85 C, 115.99 CN, 159.78 C=O.

Example 4

Encapsulation in a mono-phase system

Preparation of self-arranged poly(2'-carboxyethyl 2-cyanoacrylate) nanocapsules in a mono-phase aqueous medium.

A cooled sonication reaction vessel was filled with 50 ml of a solution of 50 mg of citric acid (citric acid monohydrate obtained from Belgorodsky Plant of Citric Acid, Belgorod, Russia) in isoosmotic dextran-based plasma substitute "Polyglukin" (Polyglukin is a Trade Mark of Krasnovarsky Plant of Medical Preparation). The mixture was cooled and titrated by H_3PO_4 to a pH of 2.5-3.2. Approximately 0.3 g of 2'-carboxyethyl 2-cyanoacrylate (prepared in Example 2) was added in portions with continuous sonication and cooling of the reaction vessel to provide a reaction temperature not higher than 30°C. When the solution became cloudy adding of 2'-carboxyethyl 2-cyanoacrylate was stopped and the mixture was sonicated for 30 min. with continuous cooling. The sonication should be stopped and the mixture cooled in the case of spontaneous heating. The nanocapsules obtained were sized by a Coulter Counter. In the remaining Examples, the nanocapsules produced were sized in the same way. The mean diameter of nanocapsules obtained was 25 nm. 95% of nanocapsules had a size of 25 nm, standard deviation 20 nm.

Example 5

Encapsulation of oxytocin in self-arranged nanocapsules formed from the 2-cyanoacrylic acid ester of polyethylene glycol 4-tert-octylphenyl ether using a mono-phase system

A cooled sonication reaction vessel was filled with 50 ml of a solution of 50 mg of citric acid in an isoosmotic salt-based solution. The mixture was cooled and titrated with phosphoric acid to a pH of 2.5-3.2. 2500 U of oxytocin was dissolved with stirring using a magnetic stirrer and 0.5 g of surface active 2-cyanoacrylic acid ester of polyethylene glycol 4-tert-octylphenyl ether monomer prepared in Example 1 was added dropwise with continuous sonication and cooling of the reaction vessel to provide a reaction temperature not higher than 30°C. When the solution became cloudy adding of monomer was stopped and the mixture was sonicated for 15 min. with continuous cooling. The sonication should be stopped and the mixture cooled in the case of overheating. After this time the suspension is transferred to a magnetic stirrer and stirred for 24 hours. The pH is then adjusted to 7.2-7.4 by the addition of 1N NaOH with continuous stirring. The nanocapsules produced were sized. The mean diameter of nanocapsules obtained was 48 nm. 95% of nanocapsules had a size of 48-49 nm, standard deviation 25 nm.

Example 6

Encapsulation of DiI in self-arranged nanocapsules formed from poly(hexadecyl 2-cyanoacrylate)

In this Example, the method according to the invention is used to encapsulate the fluorescent dye named DiI (supplied by Molecular Probes Inc., Eugene, Oregon, U.S.A.) useful for staining living cells in cell biology. DiI is insoluble in water and is a lipophylic substance accessible in the form of a solution in alcohol. DiI can be encapsulated by the use of a strong lipophilic cyanoacrylate monomer in a mono-phase aqueous-organic medium.

A cooled sonication reaction vessel was filled with 25 ml of citric buffer solution at pH=7.2-7.4, and 20 ml of alcohol containing 1 mg DiI. The mixture was cooled with stirring using a magnetic stirrer and after complete dissolution of DiI the presence of colloidal micelles was determined by a Coulter Counter. Further alcohol was added if necessary. 1 ml of alcohol solution containing 0.1 g of hexadecyl 2-cyanoacrylate prepared in Example 3 was added dropwise with continuous sonication and cooling of the reaction vessel to provide a reaction temperature not greater than 60°C. The suspension was sonicated for 30 min. with continuous cooling. The sonication should be stopped and the mixture cooled in the case of overheating. After this time the suspension was transferred to a magnetic stirrer and stirred for 24 hours. The pH was then controlled and adjusted to 7.2-7.4 by the addition of 1N NaOH with continuous stirring if necessary. The nanocapsules produced were sized. The mean diameter of the nanocapsules obtained was 37 nm. 95% of the nanocapsules had a size of 37-38 nm, standard deviation 25 nm. To estimate the yield of encapsulation, the suspension was centrifuged at 45000 r.p.m. for 4 hours and the absorbance of the solution was determined by UV spectroscopy. The estimated yield of encapsulation is 82%.

Example 7

Encapsulation of DiI in self-arranged nanocapsules formed from the 2-cyanoacrylic acid ester of polyethylene glycol 4-tert-octylphenyl ether

In this Example, a two phase aqueous system is used to encapsulate DiI. Under the appropriate conditions DiI is able to form an aqueous colloidal solution.

A cooled sonication reaction vessel was filled with a 50 ml solution of 50 mg of citric acid in an isoosmotic salt-based solution. The mixture was cooled and titrated with H_3PO_4 to a pH of 2.5-3.2. 1 mg of DiI in 1 ml of alcohol was added with sonication. The size of the micelles formed was determined by the use of a Coulter Counter and the mixture was sonicated until micelles with a size of 25-40 nm

were obtained. Then 0.1 g of the surface active 2-cyanoacrylic acid ester of polyethylene glycol 4-tert-octylphenyl ether monomer prepared in Example 1 was added dropwise with continuous sonication and cooling of the reaction vessel to provide a reaction temperature not greater than 30°C. The mixture was sonicated for 40 min. Sonication should be stopped and the mixture cooled in the case of overheating. After this time the suspension was transferred to a magnetic stirrer and stirred for 24 hours. The pH was then adjusted to 7.2-7.4 by the addition of 1N NaOH with continuous stirring. The nanocapsules produced were sized. The mean diameter of nanocapsules obtained was 115 nm. 95% of nanocapsules had a size of 115-116 nm, standard deviation 65 nm.

Example 8

Encapsulation of DiI in self-arranged nanocapsules formed from the 2-cyanoacrylic acid ester of polyethylene glycol 4-tert-octylphenyl ether

In this Example, DiI was encapsulated using an aqueous colloidal solution of two immiscible aqueous soluble polymers exemplified by the use of a dextran/polyethylene glycol (PEG) two phase system.

Preparation of the two phase system:

Dextran (10 g) and PEG (1.14 g) (supplied by Schuchardt, Munich, Federal Republic of Germany) were dissolved in 50 ml of water by mixing and heating to 80°C. After cooling 50 mg of citric acid and 0.2 ml of H_3PO_4 were added. If required, further H_3PO_4 was added to adjust the pH to 2.5-3. The mixture was then allowed to stand and the layers formed separated in a separatory funnel.

Preparation of colloidal solution and polymerisation:

1 mg DiI dissolved in 1 ml of alcohol was added dropwise to 1 ml of upper phase (primarily PEG). The solution obtained was added

to 200 ml of lower phase (primarily dextran). The mixture was then placed in a cooled sonication reaction vessel and was sonicated with continuous cooling to provide a droplet size of 60-80 nm. The size of droplets was determined by the use of a Coulter Counter as before. Sonication was continued, if necessary. The surface active monomer 2-cyanoacrylic acid ester of polyethylene glycol 4-tert-octylphenyl ether obtained in Example 1 was added dropwise with continuous sonication and cooling of the reaction vessel to provide a reaction temperature not greater than 40°C. The mixture was sonicated for 40 min. Sonication was stopped and the mixture cooled in the event of overheating. After this time the suspension was transferred to a magnetic stirrer and stirred for 24 hours. The pH was then adjusted to 7.2-7.4 by the addition of 1N NaOH with continuous stirring. The nanocapsules produced were sized. The mean diameter of nanocapsules obtained was 135 nm. 95% of the nanocapsules having a size of 135 nm, standard deviation 55 nm.

Claims: -

1. Nanocapsules comprising a polymeric shell formed of a surface active poly(alkyl cyanoacrylate) material arranged in one or more layers.

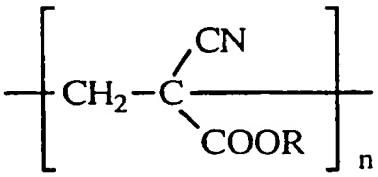
2. Nanocapsules according to Claim 1, having a diameter in the range 20-150 nm.

3. Nanocapsules according to Claim 1 or 2, wherein an aqueous phase is contained in the core defined by the polymeric shell.

4. Nanocapsules according to Claim 1 or 2, wherein a non-aqueous phase is contained in the core defined by the polymeric shell.

5. Nanocapsules according to any one of Claims 1-4, wherein an active agent is contained in the core.

6. Nanocapsules according to any preceding claim, wherein the poly(alkyl cyanoacrylate) material is a surface active poly(alkyl 2-cyanoacrylate) having the general formula:



wherein

R is $-CH_2\{CH_2\}_mCH_3$, $-CH_2\{CH_2\}_mCOOH$, $-CH_2\{CH_2\}_mCOOR'$, or $-CH_2CH_2-O\{CH_2CH_2-O\}_mR'$;

R' is $-CH_3$, $-CH_2\{CH_2\}_mCH_3$, $-C\{CH_3\}_2-CH_2-C(CH_3)_3$, or $-O-Ar$;

m has a value of from 0 to 20; and

n has a value of from 1 to 20.

7. Nanocapsules according to Claim 6, which are formed by the interfacial polymerisation of self-arranged micelles of cyanoacrylate monomers in an aqueous phase.

8. Nanocapsules according to Claim 6 or 7, which are formed by the interfacial polymerisation of a surface active cyanoacrylate monomer in the form of a colloidal solution composed of self-arranged micelles in an aqueous medium.

9. Nanocapsules according to Claim 7 or 8, wherein a mono-phase aqueous system is used.

10. Nanocapsules according to Claim 7 or 8, wherein a two phase system is used.

11. Nanocapsules according to any one of Claims 6-8 and 10, which are formed by interfacial polymerisation in a two phase aqueous polymeric emulsion.

12. Nanocapsules according to Claim 11, wherein the respective phases are present as a discrete phase and a continuous phase

13. Nanocapsules according to Claim 11 or 12, wherein the uptake of active agent in droplets of the discrete phase is promoted by adding one or more substances which cause the active agent to be expelled by the continuous phase.

14. Nanocapsules according to Claim 11 or 12, wherein the uptake of active agent in droplets of the discrete phase is promoted by adding one or more substances which cause the active agent to be attracted by said discrete phase.

15. Nanocapsules according to Claim 13 or 14, wherein the or each substance is a charged polymer.

16. Nanocapsules according to any one of Claims 5-15, wherein the active agent is a peptide or polypeptide.

17. An active agent delivery system comprising nanocapsules according to any one of Claims 1-16.

INTERNATIONAL SEARCH REPORT

Inten. ..al Application No
PCT/IE 94/00001

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K9/51

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 447 318 (L'OREAL) 18 September 1991 see claims 1-6 see page 2, line 9 - line 14 see page 14, line 12 - line 23 see page 4, line 3 - line 10	1,2,4-6, 17
X	EP,A,0 397 571 (CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE) 14 November 1990 see claims 1,6,7 see page 3, line 42 - line 49 see example b	1,3,5,6, 16,17

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
7 April 1994	15.04.94

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern al Application No
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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